Pentoxifylline protects against endotoxin-induced acute renal failure in mice

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Wang, Wei, Einath Zolty, Sandor Falk, Veena Basava, Leonid Reznikov, and Robert Schrier. Pentoxifylline protects against endotoxin-induced acute renal failure in mice. Am J Physiol Renal Physiol 291:F1090–F1095, 2006.—Acute renal failure (ARF) in septic patients drastically increases the mortality to 50–80%. Sepsis induces several proinflammatory cytokines including tumor necrosis factor-α (TNF-α), a major pathogenetic factor in septic ARF. Pentoxifylline has several functions including downregulation of TNF-α and endothelia-dependent vascular relaxation. We hypothesized that pentoxifylline may afford renal protection during endotoxemia either by downregulating TNF-α and/or by improving endothelial function. In wild-type mice, pentoxifylline protected against the fall in glomerular filtration rate (GFR; 105.2 ± 6.6 vs. 50.2 ± 6.6 μl/min, P < 0.01) at 16 h of LPS administration (2.5 mg/kg ip). This renal protective effect of pentoxifylline was associated with an inhibition of the rise in serum TNF-α (1.00 ± 0.55 vs. 7.02 ± 2.40 pg/ml, P < 0.05) and serum IL-1β (31.3 ± 3.6 vs. 53.3 ± 5.9 pg/ml, P < 0.01) induced by LPS. Pentoxifylline also reversed the LPS-related increase in renal iNOS and ICAM-1 and rise in serum nitric oxide (NO). Enhanced red blood cell deformability by pentoxifylline may have increased shear rate and upregulated eNOS. Studies were therefore performed in eNOS knockout mice. The renal protection against endotoxemia with pentoxifylline was again observed as assessed by GFR (119.8 ± 18.0 vs. 44.5 ± 16.2 μl/min, P < 0.05) and renal blood flow (0.86 ± 0.08 vs. 0.59 ± 0.05 ml/min, P < 0.05). Renal vascular resistance significantly decreased with the pentoxifylline (91.0 ± 5.8 vs. 178.0 ± 7.6 mmHg·ml⁻¹·min⁻¹, P < 0.01). Thus pentoxifylline, an FDA-approved drug, protects against endotoxemia-related ARF and involves a decrease in serum TNF-α, IL-1β, and NO as well as a decrease in renal iNOS and ICAM-1.

Sepsis is known to occur annually in 751,000 Americans and accounts for 215,000 deaths, a number equivalent to the overall deaths due to myocardial infarction (1). Moreover, sepsis is a major cause of acute renal failure (ARF) in intensive care units; sepsis-related ARF is associated with a 70–80% mortality (33, 34).

Both inflammatory and vasoactive mediators are involved in the pathophysiology of sepsis-related ARF. TNF-α plays a central role in this process. Serum levels of TNF-α are induced early in endotoxemia in humans (25). Moreover, elevated serum levels of the type I and type II receptors for TNF-α are shown to be predictive factors for ARF in patients with septic shock (12). The role of TNF-α as a pathogenetic factor in experimental ARF was supported by the observation that pretreatment with a TNF-soluble receptor (TNF-Rp55) significantly attenuated endotoxemia-related ARF (19). It has also been shown that endotoxemic ARF is caused by TNF acting directly on TNF receptor-1 in the kidney (6). TNF-α participates in the magnification of the response mediated by the release of other cytokines and active substances such as the proinflammatory nitric oxide (NO) and adhesion molecules (11, 15). Overproduction of NO by inducible nitric oxide synthase (iNOS) during endotoxemia is related to systemic vasodilation and thus hypotension (35). Meanwhile, iNOS has been implicated in endotoxemia-related ARF by inhibiting renal endothelial NO synthase (eNOS) which is an important determinant of the renal response to endotoxemia (36). The systemic vasodilation is counterbalanced by the sympathetic nervous system, renin-angiotensin-aldosterone system (RAAS), and endothelin-1, which are subsequently stimulated to maintain hemodynamic stability but may predispose the kidney to injury through renal vasoconstriction (43). Indeed, a vascular effect relating to an imbalance between renal vasoconstriction and vasodilation occurs during endotoxemic ARF (33, 43).

Pentoxifylline, a methylxanthine derivative, has been used clinically to improve erythrocyte deformability and capillary circulation for the treatment of peripheral vascular disease (3, 9). It is a nonspecific phosphodiesterase inhibitor and downregulates several proinflammatory cytokines including TNF-α (39, 46). Pentoxifylline has other functions including inhibition of platelet adherence (27, 28) and endothelia-dependent vascular relaxation (24, 29). We thus hypothesized that pentoxifylline affords renal protection by downregulating TNF-α as well as by improving endothelial function. We tested our hypothesis by using a normotensive model of endotoxemic ARF in both wild-type and eNOS knockout mice.

MATERIALS AND METHODS

Animals. The experimental protocol was approved by the Animal Ethics Review Committee at the University of Colorado Health Sciences Center. C57BL/6 and eNOS knockout mice were purchased from Jackson Laboratories (Bar Harbor, ME). The background strain of the eNOS knockout mice is C57BL/6. Male mice aged 8–10 wk were used throughout the study. Mice were maintained on a standard rodent chow and had free access to water.

Materials. Chemicals were purchased from Sigma (St. Louis, MO) unless otherwise specified.

Animal protocol. Wild-type mice were injected intraperitoneally (ip) with a 2.5-mg/kg dose of LPS (Escherichia coli 0111:B4 from LIST Biological Laboratories, Campbell, CA), which caused a dramatic decrease in glomerular filtration rate (GFR), ~75% as shown in our previous study (44). eNOS knockout mice were injected ip with a 1.0-mg/kg dose of LPS based on our previous study (45). Pentoxifylline was given ip at a dose of 150 mg/kg 30 min before LPS. The pentoxifylline dose was chosen according to publications by Irie et al. (13) and Teicher et al. (40), where 160 and 50 mg/kg × 5 over 24 h were used, respectively, in mice. The timing of the administration was chosen according to the fact that TNF-α levels peak early (~1 h) after LPS (13). Functional studies were done at 16 h after LPS injection.

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ECL assay for serum TNF-α and IL-1β. Blood was taken through cardiac puncture 16 h after LPS (2.5 mg/kg) ip injection. The ECL assay for serum TNF-α and IL-1β was performed as described previously in detail (8, 30).

Renal iNOS and ICAM-1 protein expression. Whole kidneys were homogenized in 250 mM sucrose, 25 mM imidazole, 1 mM ethylendiaminetetraacetic acid, and 1/10 volume of a protease solution consisting of 25 μg/ml antipain, 1 μg/ml aprotinin, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, 0.1 mg/ml soybean trypsin inhibitor, and 200 μM phenylmethylsulfonyl fluoride. SDS-PAGE/immunoblotting was performed on 50 μg (iNOS) or 7 μg (ICAM-1) of protein extract. Samples were electrophoresed through a 10% acrylamide gel for detection of iNOS. iNOS protein was detected using a rabbit polyclonal antibody (Upstate, Charlottesville, VA) diluted 1:1,000 in 0.1 M HCl and 0.5% Triton X-100 in 0.1 M HCl and 0.5% Triton X-100 (Biosystems, Foster City, CA).

Statistical analysis. Values are expressed as means ± SE. Multiple comparisons were assessed by ANOVA using the post hoc Newman-Keuls test. P < 0.05 was considered statistically significant.

RESULTS

Effect of pentoxifylline on serum TNF-α and IL-1β levels during endotoxemia. Blood was collected at 16 h after LPS (2.5 mg/kg) injection in wild-type mice. LPS induced a significant increase in serum TNF-α (7.02 ± 2.4 pg/ml, n = 6 vs. 0.25 ± 0.06 pg/ml, n = 6, P < 0.05; Fig. 1A) and IL-1β (53.3 ± 5.9 pg/ml, n = 11 vs. 34.9 ± 1.0 pg/ml, n = 10, P < 0.05; Fig. 1B) level compared with the controls. The administration of 150 mg/kg pentoxifylline 30 min before LPS significantly decreased serum TNF-α level (0.97 ± 0.55 pg/ml, n = 6 vs. 7.02 ± 2.4 pg/ml, n = 6, P < 0.05; Fig. 1A) as well as IL-1β level (31.3 ± 3.6 pg/ml, n = 8 vs. 53.3 ± 5.9 pg/ml, n = 11, P < 0.01; Fig. 1B).

Effect of pentoxifylline on renal iNOS protein expression and serum NO levels during endotoxemia. Renal iNOS protein expression (Fig. 2A) was significantly induced at 16 h after LPS administration (n = 6 in control [con] group and n = 6 in LPS group) which was accompanied by a significant induction in serum NO levels (230.0 ± 15.9 μM, n = 7 vs. 35 ± 10 μM, n = 6, P < 0.001; Fig. 2B). Pentoxifylline significantly attenuated the induced iNOS expression (n = 5; Fig. 2A) as well as serum NO levels (109.1 ± 24.0 μM, P < 0.01, n = 6; Fig. 2B).

Effect of pentoxifylline on renal ICAM-1 protein expression. Renal ICAM-1 protein expression was significantly induced at 16 h after LPS administration (n = 5 in control group and n = 6 in LPS group). This induction was significantly inhibited by the administration of pentoxifylline 30 min before LPS (n = 6; Fig. 3).

Effect of pentoxifylline on urine 6-keto prostaglandin F-1α and renal cortical cAMP and cGMP levels. At 16 h after LSP administration, urine 6-keto prostaglandin F-1α (27.0 ± 5.6, n = 9 vs. 10 ± 1.0 pg/ml, n = 6, P < 0.05) as well as renal cortical cGMP levels (14.5 ± 2.8 pmol/ml, n = 10 vs. 2.7 ± 0.4 pmol/ml, n = 10, P < 0.05) increased significantly compared with the control groups. However, pentoxifylline did not have any significant effects on these inductions by LPS for 6-keto prostaglandin F-1α (27.4 ± 6.9 pg/ml, n = 9 vs. 27.0 ± 5.6 pg/ml, n = 9, P = not significant) or cGMP (17.2 ± 3.8 pmol/ml, n = 10 vs. 14.5 ± 2.8 pmol/ml, n = 10, P = not significant). There was no difference in renal cortical cAMP levels among the three groups (data not shown).

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**Fig. 1.** Effect of pentoxifylline on serum TNF-α levels (number of animals in each groups is 6; A) and IL-1β levels (number of animals in 3 groups is 10, 11, and 8, respectively; B) in wild-type mice during endotoxemia. Blood was taken 16 h after LPS (2.5 mg/kg ip) injection. Levels were measured by ECL assay. Values are expressed as means ± SE.
Fig. 2. Effect of pentoxifylline on renal iNOS protein expression (A) and serum nitric oxide (NO) levels (B). Blood was taken and whole kidneys were harvested and at 16 h after LPS injection. iNOS protein expression was examined using Western blot. The number of animals in 3 groups is 6, 6, and 5, respectively. Each lane represents an individual animal. NO levels were measured using nitrate/nitrite colorimetric assay kit (the number of animals in 3 groups is 6, 7, and 6, respectively). Values are expressed as means ± SE.

Fig. 3. Effect of pentoxifylline on renal ICAM-1 protein expression. Whole kidneys were harvested and at 16 h after LPS injection. ICAM-1 protein expression was examined using Western blot. The number of animals in 3 groups is 5, 6, and 6, respectively. Each lane represents an individual animal.
Effect of pentoxifylline on renal function in wild-type mice during endotoxemia. Sixteen hours after intraperitoneal injection of 2.5 mg/kg LPS caused a significant decrease in GFR in mice as shown in our previous study. Administration of pentoxifylline 150 mg/kg 30 min before LPS injection significantly improved GFR (105.2 ± 13.3 µl/min, n = 7 vs. 50.2 ± 6.6 µl/min, n = 6, P < 0.01; Fig. 4). In the meantime, RBF (1.57 ± 0.18 ml/min, n = 7 vs. 1.60 ± 0.15 ml/min, n = 6, P = not significant) and MAP (67.3 ± 0.97 mmHg, n = 7 vs. 68.8 ± 1.7 mmHg, n = 6, P = not significant) remained unchanged. Pentoxifylline alone had no effect on GFR, MAP, or RBF compared with the baseline controls (data not shown).

Effect of pentoxifylline on renal function in eNOS knockout mice. Sixteen hours after intraperitoneal injection of 1.0 mg/kg LPS caused a significant decrease in GFR in eNOS knockout mice as shown in our previous study. Administration of pentoxifylline 150 mg/kg 30 min before LPS injection significantly improved GFR (119.8 ± 18.0 µl/min, n = 6 vs. 44.5 ± 16.2 µl/min, n = 6, P < 0.05; Fig. 5A). In the meantime, MAP decreased significantly (78.3 ± 7.6 mmHg, n = 6 vs. 105.3 ± 5.7 mmHg, n = 6, P < 0.05; Fig. 5B) and RBF increased significantly (0.86 ± 0.08, n = 7 vs. 0.59 ± 0.05 ml/min, n = 7, P < 0.05; Fig. 5C) which led to a marked decrease in renal vascular resistance (RVR; 91.0 ± 10.0 vs. 178.5 ± 17.0 mmHg·ml⁻¹·min⁻¹, P < 0.05).

DISCUSSION

Sepsis has been identified as the most common cause of ARF in intensive care units. Moreover, the combination of sepsis and ARF is associated with a very high mortality (33, 34). There is, therefore, an important need to identify potential therapeutic interventions with the potential to attenuate sepsis-related ARF. Endotoxin is known to cause much of the hemodynamic and inflammatory responses that occur in the gram-negative sepsis.

The present study was therefore undertaken to examine the effect of pentoxifylline, an FDA-approved drug, on a normotensive model of endotoxemia-related ARF in mice and its underlying mechanisms. In this model, pentoxifylline afforded significant protection against ARF in wild-type mice. The mechanisms investigated in the renal protection included the effect on serum TNF-α, IL-1β, NO levels, and renal iNOS and ICAM-1 expression.

The pathogenetic role of TNF-α has been supported by the observation that pretreatment with a TNF-soluble receptor (TNF-R55) significantly attenuated endotoxin-related ARF (19). TNF-α participates in the magnification of the response mediated by the release of other cytokines and active substances such as the proinflammatory IL-1β and NO (26). During endotoxemia and sepsis, the resultant increase in iNOS and NO is known to be associated with the arterial vasodilation and diminished systemic vascular resistance (SVR) (20, 33,
An increase in cardiac output and activation of the sympathetic and renin-angiotensin systems combine to sustain blood pressure and thereby compensate for the decrease in SVR during endotoxemia (43). The vasoconstrictor effects of ANG II, catecholamines, and increased renal sympathetic tone during endotoxemia however cause renal vasoconstriction and predispose to ARF. Support for this sequence of events is the finding that comparable α-adrenergic blockade exerts a more profound hypotensive effect in normotensive, endotoxemic mice than control mice. Moreover, renal denervation has been shown to attenuate the ARF in this normotensive, endotoxemic model of ARF (43). The rise in serum NO during endotoxemia appears to be associated with iNOS because the levels are not increased in iNOS knockout mice (19). The overproduction of NO by iNOS has been incriminated in endotoxemia-related ARF by the inhibition of renal eNOS, which is an important determinant of the renal response to endotoxia (36). The beneficial effect of eNOS on endotoxemia-related ARF was implicated by the observation that eNOS knockout mice are more susceptible to endotoxemic ARF (45). In the present study, the effect of pentoxifylline to decrease serum TNF-α was associated with diminished renal iNOS and serum NO. This finding is compatible with TNF-α as a primary cytokine in increasing iNOS.

In the present study, along with the decrease in serum TNF-α, the induction of serum IL-1β was also attenuated by pentoxifylline treatment. Thus the renal protective effect of pentoxifylline during endotoxemia in mice was associated with the inhibition of injurious proinflammatory cytokines, specifically TNF-α and IL-1β. The effect of pentoxifylline to decrease TNF-α was somewhat more pronounced than the effect on IL-1β. It must also be acknowledged that other factors may be involved in the renoprotection by pentoxifylline. The resultant beneficial effect also may have been, at least in part, mediated by the agent’s vascular effect. Specifically, pentoxifylline resulted in an impressive increase in GFR in wild-type mice treated with LPS. The ability of pentoxifylline to increase red blood cell deformability, and thus a decrease in blood viscosity (3, 9), suggested a potential vascular effect on shear-mediated increased eNOS.

The renal protection of pentoxifylline, however, occurred in the absence of eNOS in knockout mice. In fact, pentoxifylline demonstrated the same protective effect on GFR in the eNOS knockout mice as well as a significant decrease in RVR and increase in RBF. At baseline, these eNOS knockout mice have higher blood pressures and increased RVR. In the wild-type mice, the effect of pentoxifylline during endotoxemia occurred in the absence of an effect on RBF and MAP and thus may indicate a direct effect on glomerular permeability. A relaxation of the glomerular mesangium by pentoxifylline with a resultant increase in glomerular surface area could be involved. Because the eNOS knockout mice are more vasconstricted than the wild-type mice, an effect of pentoxifylline on vascular tone was more readily detected. The studies in the eNOS knockout mice were to investigate a potential role of NO generated by eNOS in the renal protective effect of pentoxifylline. The results excluded this possibility.

The effect of pentoxifylline on ICAM-1 was also examined in the present study. ICAM-1 is a member of immunoglobulin-like supergene family of adhesion molecules known to mediate adherence of polymorphonuclear neutrophil (PMN) to endothelial cells (14, 38). LPS and TNF-α both have been shown to increase the cell expression of ICAM-1 (11, 15). The pathogenic role of ICAM-1 in ARF has been demonstrated by the observations that antisense oligonucleotides (10) or antibody (16) to ICAM-1 attenuate ischemia-reperfusion injury in the rat and ICAM-1-deficient mice are protected against ischemic renal injury (17). ICAM-1 has also been shown to be a factor in endotoxemic myocardial dysfunction (31). The present study demonstrated that ICAM-1 was significantly induced in the kidney at 16 h after LPS injection and it was markedly attenuated by pretreatment of pentoxifylline. The role of ICAM in PMN accumulation has been incriminated in endotoxemic ARF (7, 22, 37), but ICAM-1 can moderate renal injury independent of PMN accumulation (31). Although detectable PMN accumulation has been observed at 16 h with this endotoxemic mouse model of ARF (44), other mechanisms may be involved. In this regard, an effect of ICAM-1 to increase cytokine production and reactive oxygen species generation has been suggested (31).

Because pentoxifylline is also a nonspecific inhibitor of cyclic 3',5'-phosphodiesterase, an increase in intracellular cAMP and cGMP might be expected. However, although renal cortical cGMP increased significantly during endotoxemia, pentoxifylline did not further increase the cGMP level. The possible contribution of renal prostacyclin to the observed decrease in RVR during pentoxifylline was also unlikely, since urine 6-keto PGF-1α, a major prostacyclin metabolite, was not changed with pentoxifylline treatment.

Although the effect of pentoxifylline in nephrotoxic renal injury secondary to cisplatin and glycerol have been studied (18, 32), there are few studies examining the effect of pentoxifylline during endotoxemia. LeMay et al. (21) demonstrate in LPS-treated rats that high doses of pentoxifylline (200 mg/kg) decreased plasma TNF-α and interleukin-6 and were antipyretic. In another study, pentoxifylline abolished LPS-induced rise in serum TNF-α and to a lesser extent IL-1β in the rat (5). Neither of these two studies however evaluated the effect of LPS or pentoxifylline on renal function. Berens et al. (4) published results indicating that pentoxifylline attenuated the LPS-related fall in intrulin clearance in the rat, but potential mechanisms were not studied. There is a study in mice receiving LPS in which pentoxifylline improved the histological damage in the kidney but renal function data were not included (2).

In summary, pentoxifylline significantly improved renal injury during endotoxemia. This protection was associated with attenuation of the induction of serum TNF-α, IL-1β, and NO as well as renal iNOS and ICAM-1 protein expression. The renal protective effect was also associated with a significant decrease in RVR in eNOS knockout mice. In conclusion, pentoxifylline, an FDA-approved drug, exhibits impressive renal protective effects in endotoxemic ARF. Therefore, a clinical trial in sepsis with pentoxifylline treatment should be considered.

**GRANTS**

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**REFERENCES**
